Demographic costs of sex allocation: hermaphrodites perform better in sparse populations

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Running title: reproductive load in simultaneous hermaphrodites

Abstract

Ophryotrocha diadema is an outcrossing, simultaneous hermaphroditic polychaete with external fertilization. In isolated pairs, mature worms take turn contributing eggs upon the condition that their partners reciprocate egg donation. In dense populations, these worms do not reciprocate. Instead, they strongly compete for mating in their preferred male role and produce few eggs. This plastic sex allocation may result in an overall different reproductive performance: mean individual reproductive output will be larger in sparse than in dense populations. We tested this hypothesis by measuring the individual reproductive output (paternal *and* maternal offspring) of worms in sparse and dense replicated populations. In dense populations, mean individual reproductive output was fourfold lower than that in sparse populations. We hypothesise that such dramatic demographic costs are potentially widespread in outcrossing simultaneous hermaphrodites with external fertilization and plastic sex allocation. The reproductive output of hermaphroditic organisms is a function of population density (i.e., the number of conspecifics) and studies on population growth and reproductive performance should take this effect into account.

Keywords: reproductive load, tragedy of the commons, population size, mating, *Ophryotrocha diadema*

Comment [CR1]: I would suggest not to include this in the keyword: it is mentioned at the very end of the study, only

Comment [MCL2]: We would prefer to leave it here: there are only few examples of this phenomenon in the literature. This paper could be more easily picked up by searching engines and cited, if this term appers in the keywords

Introduction

Simultaneous hermaphrodites have two sexual functions and partition their reproductive resources between them. Sex allocation theory predicts that they plastically adjust the proportion of resources allocated to each sex as a function of mating group size (Charnov 1982). Theory predicts that in sparse populations, where monogamous pairs may form, hermaphrodites increase their investment in eggs and limit the male function to the production of the few sperm needed to fertilize their partners' eggs. In dense populations, hermaphrodites divert resources from egg production and invest more into the male function. Experiments or observations on different hermaphrodites have tested this theory and have often found overall support for it, although the patterns of resource allocation adjustments are highly species-specific (Raimondi & Martin 1991; Trouvè et al. 1999; Schärer & Janicke 2009; Locher & Baur 2002; Hughes et al. 2002; Tan et al. 2004; Brauer et al. 2007). In some model systems, results show that sex allocation in hermaphrodites is plastic (i.e. it changes as a function of mating opportunities), as predicted by theory. For example, the hermaphroditic polychaete worms *Ophryotrocha diadema* Åkesson 1976 (Annelida: Polychaeta: Dorvilleidae) have plastic female allocation that they adjust to mating opportunities, trading-off with their investment in the male function. When mating opportunities are common (as in dense populations), worms reduce their egg production drastically and compete for mating in the male role; when mating opportunities are rare (as in sparse populations), they invest proportionally more resources in egg production and, in the absence of competitors, reduce their investment into the male function (Lorenzi et al. 2005, 2006). Sex allocation adjustments are the effect of sexual selection acting on both sexual functions in hermaphrodites (Lorenzi and Sella, 2008; Anthes et al. 2010). These adjustments are typically hermaphroditic traits, and could explain why population growth rates (as measured in dense, lab populations) are higher in gonochoric than hermaphroditic species (Prevedelli et al. 2006).

Comment [MCL3]: We prefer to start with the definition of hermaphroditism and introduce the fact that some hermaphrodites alternate between sex roles later on in the manuscript, because in the specific literature, scientists distinguish between functions and roles. E.g. each simultaneous hermaphrodite has a male function but choses whether it will play the male role depending on its partner(s) condition. We stay stuck to this definition throughout the manuscript.

Comment [MCL4]: Sperm has two plural forms either sperm or sperms but the first is more common in the specific literature

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Comment [MCL5]: We prefer to use the word adjustment because we already quoted this ability at line 52

Comment [MCL6]: We would prefer to stay with "common" . In the first review round, Referee #2 suggested the use of the word "common" (see referee #2, who suggested the fllowing: Minor point #5): " `L63 consider 'common' instead of high (and rephrase the stuff in parentheses)`"

Comment [CR7]: Explain: it is rather unclear!!

Comment [MCL8]: We clarified the text

If we assume a fixed budget for reproductive resources, we expect that hermaphrodites in large populations would use the same amount of resources for egg production than hermaphrodites in small populations, devalued of the resources diverted to increase the male function. Then we should find that hermaphrodites in large populations have a lower mean reproductive success than those in small populations. This reduced reproductive output should, in turn, affect population growth. We tested this hypothesis in the outcrossing simultaneously hermaphroditic polychaete worm *O. diadema* by measuring individual reproductive output of focal worms in sparse and dense populations.

Comment [CR9]: If mean egg production of hermaphrodites were similar in sparse and dense populations, then the amount of resources allocated to increase the male function could reduce the reproduction contribution, and hermaphrodites in dense populations would reproduce less.

Comment [MCL10]: We changed the previous sentence to clarify its meaning, following comment CR9

Material and methods

The animal model

O. diadema (Annelida, Polychaeta, Dorvilleidae) is a polychaete worm originally found in the sediments of Californian harbors. Sampling from natural populations suggests that populations have low densities (Premoli & Sella 1995). For example, only few *O. diadema* individuals were isolated among hundreds of worms of a gonochoric *Ophryotrocha* species in the Pacific Coast (pers. comm. by B. Åkesson to G.S.) and 0.1–6.6 individuals per kg^{-1} of mussel clusters were collected in the Mediterranean Sea (Simonini, pers. comm. to M.C.L.) (Schleicherová et al. 2013).

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Comment [MCL11]: We used "Californian" following the suggestion by referee #2 (see Minor comments, `L78 remove 'which was' and make it 'Californian'`)

Comment [MCL12]: A few *O. diadema* worms were found among hundreds of worms of a (single) gonochoric species.

These worms are outcrossing simultaneous hermaphrodites with external fertilization. Before maturing as hermaphrodites, they have a protandrous phase during which they can fertilize the eggs laid by hermaphrodites (Sella & Lorenzi 2003). Then, they mature as hermaphrodites, and can both fertilize their partners' eggs or lay eggs, but play one single role at each mating event. Eggs are laid in jelly cocoons and develop into larvae that leave their cocoons 8 days later and mature into simultaneous hermaphrodites in approx. 45 days. Mature hermaphrodites reproduce iteroparously for 7-10 weeks (Åkesson 1976, 1982).

In isolated pairs, worms take turns in laying cocoons of 20-25 eggs every third day (Sella 1985, 1988). When more than two worms are present, they adjust their sex allocation by investing

proportionally more resources into the male function (Lorenzi et al. 2005, 2006), mate promiscuously (Sella & Lorenzi 2000) and can share the paternity of a single egg-cocoon with other hermaphrodites (Lorenzi et al. 2013). Sex allocation adjustments are not costly in the short term (Lorenzi et al. 2008) and polychaetes sense the number of conspecifics and/or potential mates through waterborne chemical cues (Schleicherová et al. 2006, 2010; Minetti et al., 2013).

Experimental procedure

Data were gathered from focal worms. The 'focal' worms were identified through the colour of their eggs. In mature worms, eggs can be easily detected through the transparent body wall as either yellow or whitish eggs. In these worms, a dominant Y allele determines a yellow-egg phenotype, while the recessive y allele determines a white-egg phenotype (Sella and Marzona 1983). By means of this genetic marker, we can identify focal worms in a group and ascribe their progeny. The focal worms had yellow eggs and their mates white eggs.

We carried out the experiment in glass bowls filled with 10-ml artificial sea-water and kept in a thermostatic chamber at 20°C. Once a week water was replaced in the bowls and worms were fed with spinach *ad libitum*.

To obtain a sufficient number of worms for the experiment, 24 pairs of yellow-phenotype worms and 40 pairs of white ones were cultivated separately and allowed to reproduce. Their offspring supplied the virgin, newly mature, yellow- and white-phenotype worms of same age to be used for the experiment. At sexual maturity, two worms from each yellow-phenotype offspring (n = 48 worms, hereafter, 'focal worms') were randomly assigned either to sparse populations (population size = 2; the population consisting of one focal, yellow-phenotype worm and one white-phenotype partner, n = 24 replicates) or to dense populations (population size = 12, consisting of one focal, yellow-phenotype worm and 11 white-phenotype potential partners, n = 24 replicates). With such a matched-sample design, each worm in the sparse population served as a control for its sibling in the dense population.

Comment [MCL13]: We clarified the text and added details.

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Comment [CR14]: Are worms under crowdy or sparse conditions kept in same volume?

Comment [MCL15]: All bowls had a volume of 10 ml (this info is reported at line 109)

Experimental populations were checked daily for 12 days. At the first check, focal worms' body size was measured as the number of chaetigerous segments. At each check, we recorded the number of yellow (laid by focal worms) and white cocoons (laid by focal partner/s), and the number of eggs per cocoon. Adult worms were removed from the bowls on day 9.

Reproductive output of focal worms was quantified by rearing offspring until they were sexually mature. Indeed, in large populations multiple potential "fathers" were present and paternity of the progeny could be assigned to either the focal worms or one of their rivals only after the progenies were sexually mature. When these worms matured and had eggs in their coeloms, they expressed their yellow or white phenotypes and we assessed their paternity (i.e., about 45 days after egg laying). Following Åkesson (1976), this marker is neutral, since there is no difference in worm mortality rates before sexual maturity.

Comment [MCL16]: We clarified the text and added details.

The ratio between the total number of cocoons produced in sparse populations and that produced in dense populations was approx 1:2, leading to more larvae per unit of volume in the dense population bowls. Therefore, to standardize rearing conditions, on day 9 the volume of sea water was doubled in the dense population bowls.

Comment [MCL17]: We clarified the text as requested

The total reproductive output of focal worms was estimated as the number of offspring (both maternal *and* paternal offspring) that on maturity had the yellow phenotype. Focal worms without offspring were included in the calculations.

In order to control for the potentially confounding effect of differential egg-mortality in sparse and dense populations, we estimated egg mortality as the average proportion of eggs that disappeared from the cocoons in each bowl (with respect to the laid eggs).

Statistical analyses

Some replicates were excluded from calculations for various reasons (e.g., some worms died altering population size). By using related worms in sparse and dense populations, we reduced the overall variability due to genetic differences (Howell 2010). We used a linear mixed model (LMM)

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to assess the significance of the differences in reproductive output between pairs of siblings in sparse and dense populations (dependent variable: reproductive output; within-subject factor: population size; random factor: family ID; covariate: body size). Probabilities were two-tailed. Statistical analyses were performed using SPSS 20.0 statistical package (SPSS Inc, Chicago, IL).

Results

Reproductive output of focal worms

Focal worms had a dramatically lower reproductive rate in dense populations, compared to that in sparse populations, with a fourfold reduction in their reproductive output (mature maternal + paternal offspring) (Fig 1). The difference was highly significant, indicating that reproductive output in simultaneous hermaphrodites was strongly affected by population size (LMM, population size: $F_{1,35.79} = 55.557$, $P < 0.0001$; body size: $F_{1,34.99} = 4.721$, $P = 0.037$). The significant relationship between body size and reproductive output which emerged in the LMM occurred only in dense populations (Spearman's rho, in dense populations: rho = 0.496, P = 0.022; in sparse populations: rho = 0.133, P = 0.545). Body size advantage in dense populations was not associated with the female function (correlation between body size and egg production in dense populations: rho = 0.180, P = 0.460; in sparse populations: rho = 0.288, P = 0.231). This suggests that larger hermaphrodites in dense populations might have a higher reproductive output because they were more successful in the competition for the male role.

Egg mortality was not significantly different between sparse and dense populations (Wilcoxon test, Z = 73.00, total n = 20, P = 0.376), suggesting that it did not affect the results (median proportion of eggs which disappeared in sparse populations: 5.56 % *vs* 4.86% in dense populations).

Discussion

Comment [MCL18]: We clarified the sentence

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Comment [MCL19]: We deleted the sentence that should have been moved to discussion. We left a short comment at the end of the sentence, to help the reader to understand the meaning of these results..

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In this study we document that population size (i.e. the number of conspecifics) affects the reproductive output of simultaneous hermaphrodites as they produce four times more offspring in sparse than in dense populations. We interpret these results as a consequence of the fact that hermaphrodites have a plastic sex allocation which they adjust to mating opportunities. As population size increases, mating opportunities increase as well and hermaphrodites adjust their sex allocation in favour of the male function at the expense of the female function.

The reduced reproductive output of the worms in dense vs. sparse populations could be the result of uncontrolled density-dependent effects (e.g., mortality, oophagy, etc.) rather than a response to population size (e.g., the number of conspecifics). However, this hypothesis is not supported by evidence. First, egg mortality did not differ between sparse and dense populations. Second, a previous study documented that *O. diadema* worms had a higher egg production in sparse than in dense populations, irrespective of any density-dependent effects such as metabolite accumulation or encounter probability (Lorenzi et al. 2005). Furthermore, in other experiments, we simulated large population size, so that pairs of worms perceived cues as if population size were larger than two, and they reduced their egg output according to the perceived, and not the real, population size (Schleicherovà et al. 2006, 2010). All these observations support the hypothesis that worms reduce their egg output as population size increases.

It could be argued that, if worms decrease egg production in dense populations, the competition for mating as males should increase and worms with more female-biased allocation will gain higher reproductive success. Whilst this might be true in the short term, it might be disadvantageous in the long term, because fecundity often trades off with lifespan (Stearns & Hoekstra 2000). Indeed, hermaphrodites which skip the female role for long time periods live longer (Di Bona et al. 2010). Mating in sparse populations is associated with small mating groups, i.e., low numbers of partners and few or no rivals over the male role. In small mating groups, hermaphrodites invest large proportions of their reproductive resources into eggs, trade eggs with their partners and take turns in the two sexual roles (Sella, 1985; Sella & Ramella 1999). This is an evolutionary solution to the

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Comment [MCL20]: We clarified the difference between density-dependent effects and population size

Comment [MCL21]: We used the same term as above, to highlight the difference between density-dependent effects and population size

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conflict on sex roles, since both partners prefer to play the cheaper male role than the expensive female role (Leonard 1993, 2005, 2006; Di Bona et al. 2010). In natural contexts, outcrossing hermaphrodites may be constrained to monogamous mating regimes when they live in very sparse populations, as *O. diadema* (Sella & Ramella 1999; Simonini, personal communication). In other hermaphroditic species, the sizes of the populations are large but hermaphrodites are trapped in monogamous mating regimes by other life-history traits. For example, the serranid fish, *Hypoplectrus nigricans* is an outcrossing hermaphrodite which mates monogamously (Fischer 1980). Here, monogamy is constrained by the short spawning period (few hours per day), which reduces the chances that paired partners desert: reproductive gains from deserting the partner may be low, if most partners are paired.

In present study, worms in dense populations reduced their reproductive output to less than 30% when compared to worms in sparse populations. Similarly, *Plasmodium chabaudi* adjust their sex allocation in response to the presence of unrelated conspecifics. Reece et al. (2008) directly manipulated mating-group sex ratio of these malaria parasites and measured the resulting reproductive output as the number of zygotes produced. As predicted by sex allocation theory, mating output was maximized at intermediate sex ratios, indicating that sex allocation in this malaria parasite is likely to be under stabilizing selection and reproductive output was maximized at female-biased sex ratios.

Overall, our study shows that the potential individual advantages in fitness due to opportunistic sex allocation are countered at the population level when populations are dense; opportunistic sex allocation is advantageous to the individual, but disadvantageous to the population, whose reproductive rate declines. Accordingly, Prevedelli et al. (2006) found that dense populations of hermaphrodites had a demographic disadvantage compared to gonochorists. Here, we highlight that the demographic disadvantage of hermaphrodites is mainly due to their adaptive ability to adjust their sex allocation to mating group size and, ultimately, to population size. In this perspective, our study is an example of the tragedy of the commons (Hardin 1968), where traits which are

Comment [MCL22]: We changed the term using the words used by Reece et al use in their Nature paper.

Comment [CR23]: A bit confused. To be rephrased.

Comment [MCL24]: We added the explanation

advantageous at the individual level reduce population fitness. For example, strong cannibalism of larvae and pupae by adult flour beetles is adaptive at individual level but impairs population growth (Wade 1977). Similarly, hyperaggressive water-strider males gain a slightly higher mating success than less aggressive males but reduce overall group mating in their pond (Chang & Sih 2013). More specifically, sexual selection can diminish population reproductive rates of *Drosophila* populations by imposing a "reproductive load" (Holland & Rice 1999). The reproductive load highlighted in *Drosophila* was caused by antagonist sexual selection and intersexual conflicts inherent to promiscuity. Similarly, sex allocation adjustments are promoted by sexual selection acting on the two sexes of simultaneous hermaphrodites (Lorenzi & Sella 2008; Anthes et al. 2010; Leonard 2013).

We highlight that the demographic advantage of hermaphroditism in sparse populations (relative to dense populations) is the bare outcome of sex allocation adjustments in hermaphrodites where the two sexual functions interfere with each other and resources are traded off between the male and female function (Lorenzi et al. 2006). Therefore, we expect that the results we obtained here could be obtained in other hermaphroditic systems as well, where the two sexual functions act in opposition and resources are partitioned between the male and the female function on the basis of population size. We hypothesise that such dramatic demographic costs of sex allocation are potentially widespread in outcrossing simultaneous hermaphrodites with external fertilization. If the reproductive output of hermaphroditic organisms is a function of population size, population growth studies (and their practical applications) should take the effect of sex allocation into account.

Comment [MCL25]: This is the term used by Chang and Sih 2013

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Acknowledgements

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372 Figure legend

373 Figure 1. The reproductive output of focal worms (maternal and paternal offspring) in sparse and

374 dense populations (mean ± s.e.).